Docket No. 62036-PCT (51588)

IN THE U.S. PATENT AND TRADEMARK OFFICE

APPLICANT:	THE GENERAL HOSPITAL CORPORATION
INVENTOR:	Mark C. Poznansky, et al
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FOR:	FUGETACTIC PROTEINS, COMPOSITIONS AND METHODS OF USE

COPY

Box PCT
Commissioner for Patents
Washington, D.C. 20231

Sir:

AMENDMENT UNDER PCT ARTICLE 34

Pursuant to the terms of PCT Article 34, Applicant respectfully requests that the above-identified application be amended as follows (deletions shown by strike through, additions shown underlined):

IN THE SPECIFICATION:

Please amend pages 9, 10, and 19-21 as presented in the addendum to the instant Article 34 amendment.

Amended pages 9, 10, and 19-21 are submitted to show the insertion of sequence identification numbers. On page 19, the deleted text at lines 19-22 has been replaced by the recitation of "may or may not" at line 15. On pages 20-21, unnecessary sequence identification numbers have been deleted. No new matter has been added.

Further, enclosed with this document are replacement sheets (substitute sheets of pages 9, 10, 10A, and 19-21) for the originally filed pages 9, 10 and 19-21 of specification that have been amended as set forth above.

It is believed that this submission is in compliance with PCT Article 34, PCT Rules 10 and 11 and 37 CFR 1.485 which require that every original sheet of the application which is changed by an Amendment must be replaced by a substitute sheet(s) in the course of Amendment under PCT Article 34.

Entry of this Amendment Under PCT Article 34 prior to the Preliminary Examination of this application in the PCT Chapter II phase of this prosecution, therefore, is respectfully requested.

Respectfully submitted,

18 April 2005

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embodiments methods are provided which can further include the administration of a second fugetactic or anti-fugetactic agent.

Each of the limitations of the invention can encompass various embodiments of the invention. It is, therefore, anticipated that each of the limitations of the invention involving any one element or combinations of elements can be included in each aspect of the invention.

These and other aspects of the invention, as well as various advantages and utilities, will be more apparent with reference to the detailed description of the preferred embodiments.

Brief Description of the Drawings

Fig. 1 describes the amino acid sequences of HSP 90 (SEQ ID NOS 1-3), HSP 84 (SEQ ID NOS 4-5), HSP 86 (SEQ ID NO: 6), HSP 60 (SEQ ID NO: 7) and L-plastin (SEQ ID NO: 8).

Fig. 2 provides the results of a transmigration assay using 1 in 2, 1 in 10 and 1 in 100 dilutions of EL4 24-hour conditioned media (EL4CM24).

Fig. 3 provides the results of a transmigration assay using negative gradients of heat inactivated or proteinase K digested EL4 24-hour conditioned media (EL4CM24) (1 in 2, 1 in 10 and 1 in 100 dilutions).

Fig. 4 provides the results of a transmigration assay using negative gradients of EL4 24-hour conditioned media (EL4CM24) with pertussis toxin treated murine lymphocytes and radicicol and Geldanamycin treated EL4CM24 (1 in 2, 1 in 10 and 1 in 100 dilutions).

Fig. 5 provides the results of an *in vivo* study of the migration of immune cells using EL4 24-hour conditioned media (EL4CM24).

Fig. 6 provides the results of EL4 24-hour conditioned media (EL4CM24) (I0.5 and HSF) run on SDS PAGE.

Fig. 7 provides the results of the ion exchange chromatography of the EL4 24-hour conditioned media (EL4CM24).

Fig. 8 provides the results of a transmigration assay using EL4 24-hour conditioned media (EL4CM24) heat shocked at 42°C and treated with Brefeldin A.

Fig. 9 provides the mass peaks from the mass spectrometry analysis of a fraction of EL4 24-hour conditioned media (EL4CM24) that contained a protein of about 84/86 kDa.

Fig. 10 provides the mass peaks from the mass spectrometry analysis of a fraction of EL4 24-hour conditioned media (EL4CM24) that contained a protein of about 94 kDa.

Fig. 11 provides the mass peaks from the mass spectrometry analysis of a fraction of EL4 24-hour conditioned media (EL4CM24) that contained a protein of about 65 kDa.

Fig. 12 provides the MS-Fit and MS-Tag search results of a component protein of about 84 and 86 kDa (SEQ ID NOS 9-45, 121, and 46-51, respectively in order of appearance).

Fig. 13 provides the MS-Fit search results of a component protein of about 94 kDa (SEQ ID NOS 52-72, respectively in order of appearance).

Fig. 14 provides the MS-Fit and MS-Tag search results of a component protein of about 65 kDa (SEQ ID NOS 74-106, 121, and 107-118, respectively in order of appearance).

Fig. 15 provides the sequence alignment of human HSP 90-β (SEQ ID NO: 119) and mouse HSP protein 84 (SEQ ID NO: 4).

Fig. 16 provides the sequence alignment of HSP 84 (SEQ ID NO: 4) and HSP 86 (SEQ ID NO: 120), both from the mouse.

Detailed Description of the Invention

It has now been discovered, according to the invention, that tumor cells elaborate both chemokines and other chemokinetically active substances which evoke a fugetactic or chemorepellent response from immune cells, thereby allowing the neoplastic cells to evade recognition and destruction by the host immune system. Using *in vitro* and *in vivo* assays it has now been demonstrated that culture supernatant (i.e., conditioned media) from the EL4 cell line has the ability to repel lymphocytes (i.e., to induce fugetaxis). It has been further shown that migration of lymphocytes away from EL4 24-hour conditioned media (EL4CM24) was diminished by heat inactivation and proteinase digestion of the conditioned media as well as with the use of the specific inhibitors (pertussis toxin and radicicol).

invention the thymoma cell isolate is a substantially pure polypeptide. The term "substantially pure" means that the protein(s) or polypeptide(s) is essentially free of other substances with which it (they) may be found in nature or *in vitro* systems, to an extent practical and appropriate for their intended use. Substantially pure polypeptides may be produced by techniques well known in the art. Because an isolated protein may be admixed with a pharmaceutically acceptable carrier in a pharmaceutical preparation, the protein may comprise only a small percentage by weight of the preparation. The protein is nonetheless isolated in that it has been separated from many of the substances with which it may be associated in living systems, i.e. isolated from certain other proteins.

As stated above, the fugetactic agents include HSPs and L-plastin. These proteins have been discovered to possess fugetactic activity, according to the invention. It has been discovered that eluted fractions of an EL4 24-hour conditioned medium (EL4CM24) comprise proteins having amino acid sequences in common with HSPs and L-plastin. These amino acid sequences are provided in Figs. 12-14. Therefore, the fugetactic agents provided herein include proteins, which include the amino acid sequences depicted in Figs. 12-14 (set forth as SEQ ID NOs:9-118 , which may or may not include both end residues). Fugetactic agents also include proteins which comprise a portion of these sequences ~~(e.g. the amino acid sequences set forth as SEQ ID NOs:9A-118A which are the same as SEQ ID NOs:9-118 but lack either or both of the end residues, which are denoted by parenthesis)~~. The fugetactic agents of the invention, therefore, can comprise an amino acid sequence that is any one of SEQ ID NOs:1-8. The fugetactic agents of the invention can be HSP90α (Fig. 1, SEQ ID NO:3), HSP90β (Fig. 1, SEQ ID NOs:1 and 2) or L-plastin (Fig. 1, SEQ ID NO:8).

Table 1

Peptide Sequence	Fig. No.
(K)VTISNR(L) SEQ ID NO:9~~, 9A~~	12
(R)ALLFIPR(R) SEQ ID NO:10~~, 10A~~	12
(K)FYEAFSK(N) SEQ ID NO:11~~, 11A~~	12
(K)IDIIPNPQER(T) SEQ ID NO:12~~, 12A~~	12
(K)HFSVEGQLEFR(A) SEQ ID NO:13~~, 13A~~	12
(R)GVVDSEDLPLNISR(E) SEQ ID NO:14~~, 14A~~	12
(R)YHTSQSGDEMTSLSEYVSR(M) SEQ ID NO:15~~, 15A~~	12
(K)SIYYITGESKEQVANSAFVER(V) SEQ ID NO:16~~, 16A~~	12
(K)VTISNR(L) SEQ ID NO:17~~, 17A~~	12
(R)ALLFIPR(R) SEQ ID NO:18~~, 18A~~	12
(K)FYEAFSK(N) SEQ ID NO:19~~, 19A~~	12
(K)IDIIPNPQER~~T~~(T) SEQ ID NO:20~~, 20A~~	12
(K)HFSVEGQLEFR(A) SEQ ID NO:21~~, 21A~~	12
(R)GVVDSEDLPLNISR(E) SEQ ID NO:22~~, 22A~~	12
(R)YHTSQS-DEMTSLSEYVSR(M) SEQ ID NO:23~~, 23A~~	12
(K)SIYYITGESKEQVANSAFVER(V) SEQ ID NO:24~~, 24A~~	12
(K)VTISNR(L) SEQ ID NO:25~~, 25A~~	12
(R)ALLFVPR(R) SEQ ID NO:26~~, 26A~~	12
(K)FYEAFSK(N) SEQ ID NO:27~~, 27A~~	12
(K)IDILPNPQER(T) SEQ ID NO:28~~, 28A~~	12
(K)HESVEGQLEFR(A) SEQ ID NO:29~~, 29A~~	12
(R)GVVDSEDLPLNISR(E) SEQ ID NO:30~~, 30A~~	12
(R)YHTSQSGDEMTSLSEYVSR(M) SEQ ID NO:31	12
(K)SIYYITGESKEQVANSAFVER(V) SEQ ID NO:32~~, 32A~~	12
(K)VTISNR(L) SEQ ID NO:33~~, 33A~~	12
(R)ALLFIPR(R) SEQ ID NO:34~~, 34A~~	12
(K)FYEAFSK(N) SEQ ID NO:35~~, 35A~~	12

Peptide Sequence	Fig. No.
(K)HFSVEGQLEFR(A) SEQ ID NO:36~~, 36A~~	12
(R)GVVDSEDLPLNISR(E) SEQ ID NO:37~~, 37A~~	12
(R)YMTSQSGDEMTSLSEYVSR(M) SEQ ID NO:38~~, 38A~~	12
(K)SIYYITGESKEQVANSAFVER(V) SEQ ID NO:39~~, 39A~~	12
(K)IDILPNPQER(T) SEQ ID NO:40~~, 40A~~	12
(K)IDILPNPQER(T) SEQ ID NO:41~~, 41A~~	12
(K)IDIIPNPQER(T) SEQ ID NO:42~~, 42A~~	12
(K)IDILPNPQER(T) SEQ ID NO:43~~, 43A~~	12
(K)IDILPNPQER(T) SEQ ID NO:44~~, 44A~~	12
(K)IDIIPNPQER(T) SEQ ID NO:45~~, 45A~~	12
(R)ALLFVPR(R) SEQ ID NO:46~~, 46A~~	12
(R)ALLFYPR(R) SEQ ID NO:47~~, 47A~~	12
(K)AILFVPR(R) SEQ ID NO:48~~, 48A~~	12
(R)ALLFVPR(R) SEQ ID NO:49~~, 49A~~	12
(R)ALLFVPR(R) SEQ ID NO:50~~, 50A~~	12
(K)AILFVPR(R) SEQ ID NO:51~~, 51A~~	12
(K)VLTFYR(K) SEQ ID NO:52~~, 52A~~	13
(K)NTVQGFKR(F) SEQ ID NO:53~~, 53A~~	13
(K)VLATAFDTTLGGR(K) SEQ ID NO:54~~, 54A~~	13
(K)NAVEEYVYEMR(D) SEQ ID NO:55~~, 55A~~	13
(R)AGGIETIANEYSDR(C) SEQ ID NO:56~~, 56A~~	13
(R)EFSITDVVPYPISLR(W) SEQ ID NO:57~~, 57A~~	13
(R)WNSPAEEGSSDCEVFPK(N) SEQ ID NO:58~~, 58A~~	13
(K)VLTFYR(K) SEQ ID NO:59~~, 59A~~	13
(K)NTVQGFKR(F) SEQ ID NO:60~~, 60A~~	13
(K)QVYVDKLAELK(S) SEQ ID NO:61~~, 61A~~	13
(K)VLATAFDTTLGGR(K) SEQ ID NO:62~~, 62A~~	13

Peptide Sequence	Fig. No.
(K) NAVEEYVYEMR (D) SEQ ID NO:63, 63A	13
(R) AGGIETIANEYSDR (C) SEQ ID NO:64, 64A	13
(R) EFSITDVVPYPISLR (W) SEQ ID NO:65, 65A	13
(K) VLTFYR (K) SEQ ID NO:66, 66A	13
(K) NTVQGFKR (F) SEQ ID NO:67, 67A	13
(K) QVYVDKLAELK (S) SEQ ID NO:68, 68A	13
(K) VLATAFDTTIGGR (K) SEQ ID NO:69, 69A	13
(K) NAVEEYVYEMR (D) SEQ ID NO:70, 70A	13
(R) AGGIETIANEYSDR (C) SEQ ID NO:71, 71A	13
(R) EFSITDVVPYPISLR (W) SEQ ID NO:72, 72A	13
(K) VFHGLK (S) SEQ ID NO:74, 74A	14
(K) YAISMAR (K) SEQ ID NO:75, 75A	14
(R) VNKPPVPK (L) SEQ ID NO:76, 76A	14
(K) LSPEELLLR (W) SEQ ID NO:77, 77A	14
(K) IKVPVDWNR (V) SEQ ID NO:78, 78A	14
(R) QFVTATDVVR (G) SEQ ID NO:79, 79A	14
(R) NWMNSLGVNPR (V) SEQ ID NO:80, 80A	14
(K) MINLSVPDTIDER (T) SEQ ID NO:81, 81A	14
(R) VYALPEDLVEVNPK (M) SEQ ID NO:82, 82A	14
(K) FSLVGIAGQDLNEGNR (T) SEQ ID NO:83, 83A	14
(K) GDEEGIPAVVIDMSGLR (E) SEQ ID NO:84, 84A	14
(K) VFHGLK (S) SEQ ID NO:85, 85A	14
(K) YAISMAR (K) SEQ ID NO:86, 86A	14
(R) VNKPPYPK (L) SEQ ID NO:87, 87A	14
(K) LSPEELLLR (W) SEQ ID NO:88, 88A	14
(K) IKVPVDWMR (V) SEQ ID NO:89, 89A	14
(R) QFVTATDVVR (G) SEQ ID NO:90, 90A	14
(R) NWMNSLGVNPR (V) SEQ ID NO:91, 91A	14

Peptide Sequence	Fig. No.
(K) MINLSVPDTIDER (T) SEQ ID NO:93, 93A	14
(R) VYALPEDLVEVNPK (M) SEQ ID NO:94, 94A	14
(K) ESLVGIAGQDLNEGNR (T) SEQ ID NO:95, 95A	14
(K) GDEEGIPAVVIDMSGLR (E) SEQ ID NO:96, 96A	14
(K) VFHGLK (T) SEQ ID NO:97, 97A	14
(K) YAISMAR (K) SEQ ID NO:98, 98A	14
(R) VNKPPYPK (L) SEQ ID NO:99, 99A	14
(K) LSPEELLLR (W) SEQ ID NO:100, 100A	14
(K) IKVPVDWNR (V) SEQ ID NO:101, 101A	14
(R) QFVTATDVVR (G) SEQ ID NO:102, 102A	14
(R) NWMNSLGVNPR (V) SEQ ID NO:103, 103A	14
(K) MINLSVPDTIDER (T) SEQ ID NO:104, 104A	14
(R) VYALPEDLVEVNPK (M) SEQ ID NO:105, 105A	14
(K) GDEEGIPAVVIDMSGLR (E) SEQ ID NO:106, 106A	14
(R) NWMNSLGVNPR (V) SEQ ID NO:107, 107A	14
(R) NWMNSLGVNPR (V) SEQ ID NO:108, 108A	14
(R) NWMNSLGVNPR (V) SEQ ID NO:109, 109A	14
(R) NWMNSLGVNPR (V) SEQ ID NO:110, 110A	14
(R) NWMNSLGVNPR (V) SEQ ID NO:111, 111A	14
(R) NWMNSLGVNPR (V) SEQ ID NO:112, 112A	14
(R) NWMNSLGVNPR (V) SEQ ID NO:113, 113A	14
(R) NWMNSLGVNPR (V) SEQ ID NO:114, 114A	14
(R) NWMNSLGVNPR (V) SEQ ID NO:115, 115A	14
(R) NWMNSLGVNPR (V) SEQ ID NO:116, 116A	14
(R) NWMNSLGVNPR (V) SEQ ID NO:117, 117A	14
(R) NWMNSLGVNPR (V) SEQ ID NO:118, 118A	14